Name of Organization: University of Akron

Type of Organization: College or University

Contact Information: Dr. Teresa Cutright

Civil Engineering

The University of Akron

Akron OH 44325

Phone: (330) 972 - 4935 **Extension:**

Fax: (330) 972 - 6020

E-Mail: TCUTRIGHT@UAKRON.EDU

Project Title: Multienzymatic System for the Minimization of Waste Solvents

Project Category: Pollution Prevention and Reduction - BNS

Rank by Organization (if applicable): 0

Total Funding Requested ($): 121,545 **Project Duration:** 2 Years

Abstract:

The quality of the Great Lakes ecosystems are threatened by both existing problems as well as future contamination. One of the most prominant new souces of contamination stems from solvent emissions and releases from nearby industries. Biocatalysis has been demonstrated to be an important approach to environmental remediation and pollution prevention of waste organics. Compared to whole-cell bioprocessing, alternative enzymatic biotransformation can afford a number of advantages which include faster reactions and the ability to tolerate elevated organic concentrations. However, previous research efforts on enzymatic biodegradation of organic wastes have been limited to the use of individual enzymes, which usually achieve only partial degradation with products that may retain much toxicity. Accordingly, the objectives of proposed research include: (1) fundamental studies of in vitro multi-enzymatic catalysis mimicking microorganisms that are capable of degrading organic pollutants; and (2) activation and stabilization of the multienzymic system to extend the enzymes effective lifetime. It is expected that such biocatalytic systems will lead to degradation of organic contaminants equivalent to that achieved by microorganisms. However the use of enzymes will dramatically reduce the remediation time and can be applied at harsh conditions, such as wastewater with very high concentration of organic pollutants, that are often toxic enough to inhibit microorganisms.

Geographic Areas Affected by the Project

States:

- ☐ Illinois
- ☐ Indiana
- ☒ Michigan
- ☐ Minnesota
- ☐ New York
- ☐ Pennsylvania
- ☐ Wisconsin
- ☒ Ohio

Lakes:

- ☐ Superior
- ☐ Huron
- ☐ Michigan
- ☒ Erie
- ☐ Ontario
- ☐ All Lakes

Geographic Initiatives:

☐ Greater Chicago ☒ NE Ohio ☐ NW Indiana ☐ SE Michigan ☐ Lake St. Clair

Primary Affected Area of Concern: Cuyahoga River, OH

Other Affected Areas of Concern: Lake Erie

For Habitat Projects Only:

Primary Affected Biodiversity Investment Area: Not Applicable

Other Affected Biodiversity Investment Areas:

Problem Statement:

Problem Statement

The environmental goal of the Binational Toxics Strategy (BNS) focuses on the reduction/treatment of persistent chemicals in Great Lakes waterways and sediments. Although this is both a commendable and daunting task, it will only solve part of the problem. The Great Lakes are also subject to new contaminants originating from accidental spills and industrial emissions. These new contaminants pose just as severe a threat as the existing problems posed by the compounds listed by the BNS. One such threat stems from waste solvents. The generation of solvent waste has become so wide spread that it has been classified as one of the most critical environmental problems facing Ohio [OEPA, 1999]. For example, the 1997 Toxic Release Inventory reported that over 5,165,000 pounds of waste solvents (including 956,083 pounds of TCE) were released as fugitive air emissions and discharged to surface waters [OEPA, 1997]. In Summit County alone, there were 230,000 pounds of toluene and 1-chloro-1,1-difluoroethane released to surface waters. Depending on the source location and transport (mode, media, and direction) issues, these solvents will end up in Lake Erie and other waterways. The goal of this project will to decrease the amount of solvent(s) that can potentially contaminate Lake Erie. This will be achieved by using a novel biocatalysis process, which purifies the waste stream in-situ thereby minimizing the amount of waste generated and ultimately released. Although the project will be developed to directly address Lake Erie concerns, it can be easily adapted for protecting the other Great Lakes as well.

Proposed Work Outcome:

Proposed Work

Previous research efforts on both intact microbial cells and free enzymes for biodegradation of organic wastes have been limited to the use of either a single microbe or enzyme. Single microbes/enzymes typically only achieve a partial degradation with byproducts that may retain a high degree of toxicity. Accordingly, the objectives of proposed research include: (1) fundamental studies of microbial consortiums and in vitro multi-enzymatic catalysis that are capable of degrading aqueous based organic pollutants; and (2) activation and stabilization of the multienzymatic system via polyvalent binding of proteins to solid supports for biodegradation of multiple solvents. To achieve these objectives, the following three phases of activity must be completed.

Phase I: The first Phase involves the microbial degradation of waste solvents and activation of enzymes in aqueous media. At this stage, the microbial consortium will be developed by standard subculturing techniques. The consortium will be used to assess the effectiveness of intact cells for degrading the organic solvent. Phase I will also be used to harvest and isolate the enzymes needed for developing a multi-enzyme system.

Phase II: The second phase of the project will focus on the development and assessment of a multi-enzyme system for treating the aqueous phase waste solvent. Enzymes isolated from the microbial solution will be tested to mimic the microbial degradation of a model compound such as TCE. The resulting protein content and enzyme activity of the multi-enzyme mixture will also be examined.

Phase III: The approach briefly outlined for Phase I and II will then be repeated for a system comprised of more than one solvent (i.e., toluene and TCE). This step will be used to determine the effect of competition and inhibition on the process efficiency. It will also be used to demonstrate the versatility of the proposed process for waste minimization in many industrial applications.
Completion of the project will demonstrate, for the first time, the degradation and mineralization of organic contaminants in aqueous media via a multi-enzyme system. The proposed research will lead to a new class of enhanced bioremediation technologies that offer large-scale, simple operation, low cost and fast reactions for the treatment of organic pollutants. Treating these pollutants at the source will reduce the amount of solvents that enter into the Great Lake System.

Impact on BNS compounds
TCE was selected as a germane model compound due to its prevalence in the Northeastern solvent emissions. Furthermore, the large quantity of information regarding TCE systems that is readily available will facilitate the study as a general strategic approach for a wide range of contaminants. BNS compounds can be easily treated in the same manner as an extended future study. Preventing the introduction of solvents into the Great Lakes will significantly enhance both current and future remediation efforts of BNS compounds. This enhancement will be evident by eliminating diminished process efficiencies that result form the presence of competing compounds such as chlorinated solvents. Furthermore, preventing the introduction of the solvents into the lakes will serve to protect the biological and physical integrity of the Lakes ecosystems.

Dissemination of Results
Dissemination of the results is critical in ensuring that successful minimization solvent waste is realized. For that reason, three vehicles have been identified to inform the regulatory officials, industrial managers, engineering community, and the general public. First, the results will be presented at national scientific conferences for rapid dissemination. Long term dissemination will encompass publishing the results in the appropriate scientific journals (Water Research, Environmental Science & Technology, Pollution Prevention, etc.). The EPA Technology Transfer Center in Cleveland will also be contacted for enabling the findings to reach the industrial users in the Northeastern Ohio area.

Applicable Industries
There is a multitude of manufacturing/processing industries located in Northern Ohio and Southern Michigan that incorporate solvents within one or more of their process steps. This technology will be readily applicable to minimizing the amount of aqueous based organic contaminants that originate from these companies. The initial industries that will be targeted for introducing the new technology will be determined from sources such as the Toxic Release Inventory, CAMP Manufacturing Association, EPA's Technology Transfer Center, local professional societies etc.

Project Milestones:	Dates:
Project start	09/2000
Whole cell microbial evaluation	11/2001
Enzyme isolation and stabilization	11/2001
Multienzyme system - one solvent	03/2002
Microbial degradation of >1 solvent	06/2002
Multienzyme system >1 solvent	08/2002
Project documentation and dissemination	09/2002
Project End	09/2002

☐ Project Addresses Environmental Justice

If So, Description of How:

☐ Project Addresses Education/Outreach

If So, Description of How:

Project Budget:

	Federal Share Requested ($)	Applicant's Share ($)
Personnel:	73,240	28,000
Fringe:	7,275	0
Travel:	0	1,000
Equipment:	0	0
Supplies:	12,850	1,000
Contracts:	0	0
Construction:	0	0
Other:	0	0
Total Direct Costs:	93,365	30,000
Indirect Costs:	28,180	2,000
Total:	121,545	32,000
Projected Income:	0	0

Funding by Other Organizations (Names, Amounts, Description of Commitments):

The entire project will take three years to complete. As shown in the budget, funds have only been requested for the first two years. An OBR matching grant will be applied for to provide and additional $20,000 in matching funds. These funds will be used to for the graduate student salaries, purchase additional analytical supplies, dissemination expenditures, and offset some travel expenditures during the final year. Securement of the OBR matching grant is contingent upon the successful funding of this proposal.

Description of Collaboration/Community Based Support:

Compeletion of the project requires the collaborative efforts of two investigators - Dr. Teresa Cutright (Civil Engineering) and Dr. Ping Wang (Chemical Engineering). The local section of the American Institute of Chemical Engineers will assist with identifing the target industries that will implement the process in the future.